10/031692

JC07 Rec'd PCT/PTO 22 JAN 2002

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TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

Attorney's Docket Number: 50203/015001

U.S. Application Number:

INTERNATIONAL APPLICATION NUMBER	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP00/07023	July 21, 2000	July 22, 1999

TITLE OF INVENTION:	CERAMIDE ANALOGS, PROCESS FOR THEIR PREPARATION AND THEIR USE AS ANTITUMOR AGENTS
APPLICANTS FOR DO/EO/US:	Bruno Macchia, Aldo Balsamo, Marco Macchia, Mario Del Tacca and Romano Danesi

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1.	■ This is a FIRST submission of items concerning a filing under 35 U.S.C. § 371.
2.	□ This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. § 371.
3.	□ This is an express request to begin national examination procedures (35 U.S.C. § 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. § 371(b) and PCT Articles 22 and 39(1).
4.	■ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5.	A copy of the International Application as filed (35 U.S.C. § 371(c)(2)). ■ a. is transmitted herewith (required only if not transmitted by the International Bureau). □ b. has been transmitted by the International Bureau. □ c. Is not required, as the application was filed with the United States Receiving Office (RO/US).
6.	□ A translation of the International Application into English (35 U.S.C. § 371(c)(2).
7.	Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. § 371(c)(3)). □ a. are transmitted herewith (required only if not transmitted by the International Bureau). □ b. have been transmitted by the International Bureau. □ c. have not been made; however, the time limit for making such amendments has NOT expired. ■d. have not been made and will not be made.
8.	□ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. § 371(c)(3)).
9.	■ An oath or declaration of the inventors (35 U.S.C. § 371(c)(4)).
10.	□ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. §371 (c)(5).
11.	□ An Information Disclosure Statement under 37 C.F.R. §§ 1.97 and 1.98.
12.	□ An assignment for recording. A separate cover sheet in compliance with 37 §§ 3.28 and 3.31 is included.
13.	□ A FIRST preliminary amendment. □ A SECOND or SUBSEQUENT preliminary amendment.
14.	□ A substitute specification.
15.	□ A change of power of attorney and/or address letter.
16.	■ Other items or information: A copy of Priority Italian Appn. FI99/A000169 (22 pp.); and PCT Appn. PCT/EP00/07023 (36 pp.)

17.	■ The following fees are submitted: BASIC NATIONAL FEE (37 C.F.R. § 1.492(A)(1)-(5)): Neither international preliminary examination fee (37 C.F.R. § 1.482) nor international search fee (37 C.F.R. § 1.455(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO $ 1040.00 International preliminary examination fee (37 C.F.R. § 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO $ 890.00 International preliminary examination fee (37 C.F.R. § 1.482) not paid to USPTO but international search fee (37 C.F.R. § 1.445(a)(2)) paid to USPTO $740.00 International preliminary examination fee (37 C.F.R. § 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1) - (4) $ 710.00 International preliminary examination fee paid to USPTO (37 C.F.R. § 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) $ 100.00				$890.00	
				ENTER APPROPRIATE BASIC FEE AMOUNT =	$890.00	
				Surcharge of $130 for furnishing the oath or declaration later than □ 20 OR □ 30 months from the earliest claimed priority date (37 C.F.R. § 1.492(e)).	$	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE			
Total claims	[12] - 20 =	0	x $18		$	
Independent claims	[1] - 3 =	0	x $84		$	
Multiple dependent claims (if applicable)			+ $280		$280.00	
				TOTAL OF ABOVE CALCULATIONS =	$1,170.00	
				Reduction of 1/2 for filing by small entity, if applicable. [**Applicant claims small entity status under 37 C.F.R. § 1.27**]	$	
				SUBTOTAL =	$	
				Processing fee of $130.00 for furnishing the English translation later than □ 20 OR □ 30 months from the earliest claimed priority date (37 C.F.R. § 1.492(f)). +	$	
				TOTAL NATIONAL FEE =	$	
				Fee for recording the enclosed assignment (37 C.F.R. 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 C.F.R. §§ 3.28, 3.31). $40.00 per property. +	$	
				TOTAL FEES ENCLOSED =	$ 1,170.00	
					Amount to be refunded	$
					charged	$

■1,170 a. A check in the amount of $1,170.00 to cover the above fees is enclosed.
□ b. Please charge my Deposit Account No. 03-2095 in the amount of $ [**.**] to cover the above fees.
■ c. The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment, to Deposit Account No. 03-2095.

NOTE: Where an appropriate time limit under 37 C.F.R. §§ 1.494 or 1.495 has not been met, a petition to revive (37 C.F.R. § 1.137(a) or (b) must be filed and granted to restore the application to pending status.

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CERAMIDE ANALOGS, PROCESS FOR THEIR PREPARATION AND THEIR USE AS ANTITUMOR AGENTS

Field of the invention

The present invention concerns the ceramide analog compounds of the general formula (I) specified below, their corresponding preparation process, and their use in the preparation of pharmaceutical formulations with an antitumor effect.

State of the art

Ceramides are lipids composed of a fatty acid and sphingosine joined together by an amide link; they are generated by sphingomyelin, a sphingolipid occurring in the membranes of eukaryote cells due to the action of the enzyme sphingomyelinase, or they are synthesized by the action of the enzyme ceramide synthetase.

Sphingolipids such as sphingomyelin have always been considered as stable and metabolically inactive structural components of the membranes. It is only in the last decade that it has been demonstrated, instead, that sphingolipids have an active role in the mechanisms regulating cell response to exogenous stimuli, as well as in regulating cell growth, differentiation, transformation and adhesion.

It has also recently been demonstrated that the products of the demolition of sphingolipids, i.e. ceramides and sphingosine, play an important part in regulating the transmission mechanisms of the signals controlled by the membrane

sphingolipids (Teruyuki Sakai et al., *Exp Opin Ther Patents* [1988] 8 [12]: 1673-1682). In particular, the distinctive characteristic of these products seems to be their involvement in the antiproliferative mechanisms of cell regulation, such as cell growth inhibition, the induction of cell differentiation and programmed cell death, or apoptosis.

Apoptosis has recently been the object of numerous studies (e.g. Ross A. Kinloch et al., *TiPS*, Jan 1999 [20]: 35-42), because this phenomenon lends itself to pharmacological "manipulation": in fact, a reduction in the frequency of the onset of cell apoptosis can have severe pathological consequences and facilitate tumor growth, hence the therapeutic potential of all those compounds that are capable of stimulating apoptosis.

From in-depth studies it has emerged that the ceramides in the cell membranes act as intracellular "effectors" of apoptosis, and therefore as potential inhibitors of tumor growth.

In order to boost this capacity of the endogenous ceramides pharmacologically, the ideal strategy seems to be to develop endogenous ceramide analogs that mimic their effects, are stable in relation to metabolization of the sphingosine ceramide and have an inhibitory effect on the ceramidase in order to prevent the generation of sphingosine, which represents a factor that stimulates proliferation, starting from the endogenous sources of ceramides.

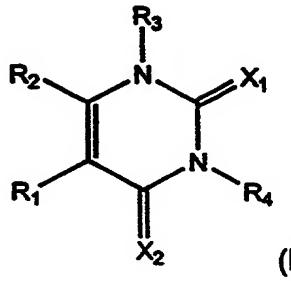
Such ceramide analogs should also have the capacity to penetrate the cell membrane.

There is consequent a need for ceramide analog compounds that are capable of crossing the cell membranes, penetrating inside the cells and mimicking the

various properties of the ceramides, and particularly that of inducing apoptosis in human cancer cells.

Summary of the invention

The Applicant has now surprisingly discovered that the ceramide analog compounds of formula (I):



(I)

wherein:

X_1 and X_2 are selected between O and S;

R_1 and R_2 are selected between $-(CH_2)_{13}CH_3$ and alkyl or alkylene groups with from 2 to 6 carbon atoms, linear or branching, unsubstituted or substituted with one or more substituents selected among aromatic, primary, secondary and tertiary aminic, quaternary ammonium, carboxylic, hydroxylic, polyoxyalkylic and ethereal groups, aminoacids, halogen atoms or saccharidic portions, providing that between R_1 and R_2 only one is always $-(CH_2)_{13}CH_3$,

R_3 and R_4 are selected between H and alkyl or alkylene groups with from 2 to 6 carbon atoms, linear or branching, unsubstituted or substituted with one or more substituents selected among aromatic, primary, secondary and tertiary aminic, quaternary ammonium, carboxylic, hydroxylic, polyoxyalkylic and ethereal groups, aminoacids, halogen atoms or saccharidic portions,

are capable of penetrating inside the biological membranes and effectively inducing apoptosis of the cancer cells.

The compounds of the general formula (I) considered in the present invention have therefore proved suitable for the preparation of pharmaceutical formulations for the treatment of tumors.

The object of the present invention is therefore represented by the compounds of the general formula (I), their corresponding preparation process, and their use in the preparation of pharmaceutical formulations for use in the treatment of tumors.

The characteristics and advantages of the compounds of the general formula (I) according to the present invention will be illustrated in detail in the following description.

DETAILED DESCRIPTION OF THE INVENTION

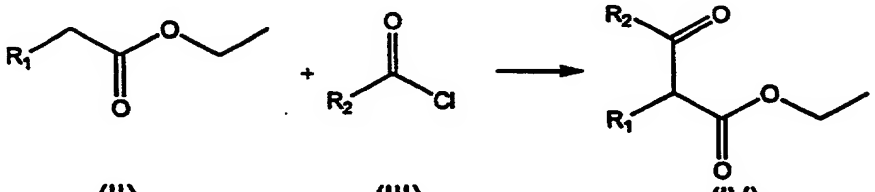
The present invention refers to the compounds of the general formula (I), as defined above. Said compounds (I) have proved capable of penetrating inside the biological membranes and effectively inducing the apoptosis of cancer cells. The following compounds have proved particularly effective and highly cytotoxic:

- compound of formula (I) where X_1= S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = ethyl, and R_3 = R_4 = H [compound (3)];
- compound of formula (I) where X_1= X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = ethyl, and R_3 = R_4 = H [compound (4)];
- compound of formula (I) where X_1= X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = *n*-propyl, and R_3 = R_4 = H [compound (6)];
- compound of formula (I) where X_1= X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = *i*-butyl, and R_3 = R_4 = H [compound (10)];

The present compounds of formula (I) can be conveniently prepared by processes well known in the art. For example, a process for the preparation of the present

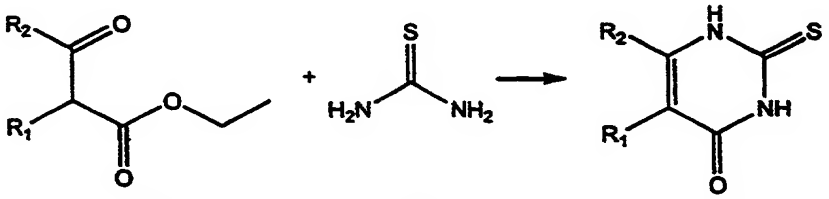
compounds of formula (I) wherein $R_3 = R_4 = H$ includes the following steps:

i) reaction of the ethyl ester (II) with acid chloride (III) to obtain the ß-ketoester of formula (IV):



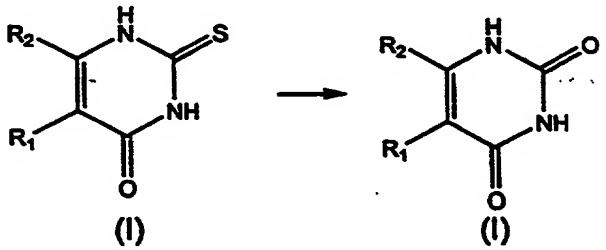
(II) (III) (IV)

ii) reaction of the ß-ketoester of formula (IV) with thiourea (V) to obtain the compound of formula (I) where $X_1 = S$, $X_2 = O$:



(IV) (V) (I)

iii) reaction compound of formula (I) where $X_1 = S$, $X_2 = O$, with refluxed chloroacetic acid to obtain the compound of formula (I) wherein $X_1 = X_2 = O$:



(I) (I)

wherein X_1, X_2, R_1 and R_2 have above-specified meanings.

Step i) of the said process is generally carried out in an organic solvent, such as THF, at a temperature of 0°C. Said reaction is preferably carried out in an inert gas atmosphere.

The reaction product of formula (IV) can be recovered from the reaction mixture by addition of a saturated NH_4Cl solution and subsequent extraction with diethyl ether.

Step ii) of the present process is carried out by means of the addition of thiourea in ethanol and sodium ethoxide on the raw reaction product coming from step i), without the need for any purification. In step ii) temperature is preferably maintained around 90°C. The reaction product is generally recovered from the reaction mixture by acidification at pH 2, e.g. by adding conc. HCl, and filtration of the resulting precipitate, which can be purified, if necessary, by washing with acetone.

The reaction product obtained in step ii) can be further purified by chromatography on silica gel, preferably using a mixture of ethyl acetate and petroleum ether in proportions of 2:1 as an eluant.

Step iii) of the process according to the above procedure is generally carried out by adding chloroacetic acid to the product coming from step ii), e.g. in the form of a 10% aqueous solution, and reflux heated. The crude residue thus obtained can then be purified by washing with absolute ethanol and then with diethyl ether.

The product coming from step iii) can be further purified by chromatography on silica gel, preferably using a mixture of ethyl acetate and hexane in proportions of 1:2 as an eluant.

The present compounds of formula (I) wherein R_3 and/or R_4 are different from H, can be prepared from the ß-ketoester of formula (IV) or from the compounds of formula (I) wherein $R_3 = R_4 = H$, obtained for example as explained above, by means of well-known processes.

Other processes for the preparation of the present formula (I) compounds are disclosed in the examples.

The compounds of formula (I) according to the present invention can be formulated with pharmaceutically acceptable excipients and/or diluents in order to prepare pharmaceutical formulations suitable for the treatment of tumor pathologies.

The following examples are given as a partial illustration of the present invention.

EXAMPLE 1

Preparation of the compound of formula (I) where X_1 = S, X_2 = O, R_2 = –$(CH_2)_{13}CH_3$, R_1 = ethyl, and R_3 = R_4 = H [compound (1)]

A solution prepared by dissolving 0.37 g of ethyl butyrate in 2 ml of anhydrous tetrahydrofuran (THF) is added drop by drop, at a temperature of 0°C and in an argon gas atmosphere, to 1.9 ml of a 2M solution of lithiodiisopropylamine (LDA) in anhydrous THF. After 30 minutes of agitation at 0°C, the reaction mixture is added to a solution obtained by dissolving 1 g of pentadecanol chloride (3.8 mmol) in 5 ml of anhydrous THF, previously cooled to 0°C. The resulting mixture is constantly agitated at room temperature for 12 hours, then added to a saturated solution of NH_4Cl. The organic phase is separated from the aqueous phase, then extracted with diethyl ether. The organic extracts are combined, washed with a saturated aqueous solution of NaCl, dried with anhydrous Na_2SO_4 and then evaporated until dry to provide a crude residue (1.20 g) composed almost exclusively of ß-ketoester (IV) where R_2 = –$(CH_2)_{13}CH_3$ and R_1 = ethyl. [^{1}H-NMR ($CDCl_3$, 200 MHz) δ 0.83-0.94 (m, 6H), 1.07 (t, 3H, *J* = 7.4 Hz), 1.15-1.36 (m, 24H), 1.81-2.02 (m, 2H), 2.11-2.57 (m, 2H), 3.34 (t, 1H, *J* = 7.3 Hz), 4.15 (q, 2H, *J* = 7.3 Hz). MS m/e 340 M^+].

The resulting crude residue (1.20 g) containing the ß-ketoester (IV) where R_2 = –

$(CH_2)_{13}CH_3$ and R_1 = ethyl, is dissolved in 20 ml of absolute ethanol and then added to 3.61 g of thiourea (47.5 mmol) and 6.47 g of sodium ethoxide (95.1 mmol). The mixture is agitated for 60 minutes at 90°C. After cooling to room temperature, the reaction mixture is filtered and the filtrate is evaporated until dry; the residue thus obtained is then restored with a mixture of water and THF in proportions of 10:1 until it has become completely soluble. The solution, cooled to 0°C, is acidified to pH 2 with conc. HCl; the precipitate that develops is filtered and washed with small quantities of acetone and provides a crude residue that is purified by chromatography on silica gel using ethyl acetate and petroleum ether in proportions of 2:1 as an eluant, finally obtaining 290 mg (0.82 mmol; yield = 26%) of the required compound of formula (I) (m.p. = 167-169°C; [^{1}H-NMR ($CDCl_3$, 200 MHz) δ 0.89 (t, 3H, *J* = 6.2 Hz), 1.09 (t, 3H, *J* = 7.4 Hz), 1.17-1.36 (m, 24H), 2.34-2.49 (m, 4H), 8.88 (br, 1H, D_2O exchangeable), 9.81 (br, 1H, D_2O exchangeable); MS m/e 352 M^+).

EXAMPLE 2

Preparation of the compound of formula (I) where $X_1 = X_2 = O$, $R_2 = -(CH_2)_{13}CH_3$, R_1 = ethyl, and $R_3 = R_4 = H$ [compound (2)]

160 mg (0.45 mmol) of the product (1) obtained as described in Example 1 are added to 11.4 ml of a 10% aqueous solution of chloroacetic acid and the mixture thus obtained is reflux heated for 12 hours. The resulting precipitate is then filtered, washed first with absolute ethanol, then with diethyl ether, to obtain a crude residue that, after purification by chromatography on silica gel using a mixture of ethyl acetate and hexane in proportions of 1:2 as an eluant, gave rise to 48 mg (0.14 mmol, yield = 32%) of the required pure compound (m.p. = 132-

134°C; [^{1}H-NMR ($CDCl_3$, 200 MHz) δ 0.87 (t, 3H, *J* = 6.2 Hz), 1.06 (t, 3H, *J* = 7.4 Hz), 1.15-1.36 (m, 24H), 2.31-2.49 (m, 4H), 9.06 (br, 1H, D_2O exchangeable), 9.89 (br, 1H, D_2O exchangeable); MS m/e 336 M^+).

EXAMPLE 3

Preparation of the compound of formula (I) wherein X_1= S, X_2 = O, R_1 = –$(CH_2)_{13}CH_3$, R_2 = ethyl, and R_3 = R_4 = H [compound (3)]

A solution obtained by dissolving 1 g of ethyl palmitate (3.52 mmol) in 3 ml of anhydrous THF is added drop by drop, at a temperature of 0°C in an argon gas atmosphere, to 2.1 ml of a 2M solution of lithiodiisopropylamine (LDA) in anhydrous THF. After 30 minutes of agitation at 0°C, the reaction mixture is added to a solution obtained by dissolving 2.39 g (4.23 mmol) of propionyl chloride in 5 ml of anhydrous THF. The resulting mixture is constantly agitated at room temperature for 12 hours, then added to a saturated solution of NH_4Cl. The organic phase is separated from the aqueous phase, then extracted with diethyl ether. The organic extracts are combined, washed with a saturated aqueous solution of NaCl, dried with anhydrous Na_2SO_4 and then evaporated until dry to provide a crude residue (1.31 g) composed almost exclusively of ß-ketoester (IV) where R_1 = –$(CH_2)_{13}CH_3$ and R_2 = ethyl. [^{1}H-NMR ($CDCl_3$, 200 MHz) δ 0.79-0.92 (m, 6H), 1.11 (t, 3H, *J* = 7.6 Hz), 1.17-1.39 (m, 24H), 1.48-1.62 (m, 2H), 2.26 (q, 2H, *J* = 7.6 Hz), 3.36 (t, 1H, *J* = 7.3 Hz), 4.15 (q, 2H, *J* = 7.2 Hz); MS m/e 340 M^+].

1.31 g of the resulting crude residue containing the ß-ketoester (IV) where R_1 = –$(CH_2)_{13}CH_3$ and R_2 = ethyl, is dissolved in 20 ml of absolute ethanol and then added to 4.01 g of thiourea (52.8 mmol) and 7.18 g of sodium ethoxide (105.6 mmol). The mixture is agitated for 60 minutes at 90°C. After cooling to room

temperature, the reaction mixture is filtered and the filtrate is evaporated until dry; the residue thus obtained is then treated with a mixture of water and THF in proportions of 10:1 until it has become completely soluble. The solution is cooled to 0°C and acidified to pH 2 with conc. HCl; the precipitate that develops due to acidification is filtered and washed with small quantities of acetone and provides a crude residue that is purified by chromatography on silica gel using ethyl acetate and petroleum ether in proportions of 2:1 as an eluant, finally obtaining 310 mg (0.88 mmol; yield = 25%) of a product that coincides with the required pure compound 3 (m.p. = 100-102°C; [^{1}H-NMR ($CDCl_3$, 200 MHz) δ 0.88 (t, 3H, J = 6.4 Hz), 1.01 (t, 3H, J = 7.4 Hz), 1.18-1.38 (m, 24H), 2.35 (t, 2H, J = 7.4 Hz), 2.48 (q, 2H, J = 7.6 Hz), 9.08 (br, 1H, D_2O exchangeable), 9.73 (br, 1H, D_2O exchangeable); MS m/e 352 M^+).

EXAMPLE 4

Preparation of the compound of formula (I) wherein $X_1 = X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = ethyl, and $R_3 = R_4 = H$ [compound (4)]

160 mg (0.45 mmol) of the compound (3) obtained as described in Example 3 are added to 11.4 ml of a 10% aqueous solution of chloroacetic acid and the mixture thus obtained is reflux heated for 12 hours. The resulting precipitate is then filtered, washed first with absolute ethanol, then with diethyl ether, to obtain a crude residue that, after purification by chromatography on silica gel using a mixture of ethyl acetate and hexane in proportions of 1:2 as an eluant, gave rise to 57 mg (0.17 mmol, yield = 38%) of the compound (4) (m.p. = 110-112°C; [^{1}H-NMR ($CDCl_3$, 200 MHz) δ 0.89 (t, 3H, J = 6.4 Hz), 1.02 (t, 3H, J = 7.4 Hz), 1.12-1.42 (m, 24H), 2.34 (t, 2H, J = 7.2 Hz), 2.49 (q, 2H, J = 7.6 Hz), 9.15 (br, 1H, D_2O

exchangeable), 9.53 (br, 1H, D_2O exchangeable); MS m/e 336 M^+).

EXAMPLE 5

Preparation of the compound of formula (I) wherein X_1 = S, X_2 = O, R_1 = –$(CH_2)_{13}CH_3$, R_2 = *n*-propyl, and R_3 = R_4 = H [compound (5)]

Compound (5) was prepared following a procedure similar to the one described in Example 3, obtaining a product which resulted in: MS m/e 366 M^+.

EXAMPLE 6

Preparation of the compound of formula (I) wherein X_1 = X_2 = O, R_1 = –$(CH_2)_{13}CH_3$, R_2 = *n*-propyl, and R_3 = R_4 = H [compound (6)]

Compound (6) was prepared following a procedure similar to the one described in Example 4, obtaining a product which resulted in: MS m/e 350 M^+.

EXAMPLE 7

Preparation of the compound of formula (I) wherein X_1 = S, X_2 = O, R_1 = –$(CH_2)_{13}CH_3$, R_2 = *n*-butyl, and R_3 = R_4 = H [compound (7)]

Compound (7) was prepared following a procedure similar to the one described in Example 3, obtaining a product which resulted in: MS m/e 380 M^+.

EXAMPLE 8

Preparation of the compound of formula (I) wherein X_1 = X_2 = O, R_1 = –$(CH_2)_{13}CH_3$, R_2 = *n*-butyl, and R_3 = R_4 = H [compound (8)]

Compound (8) was prepared following a procedure similar to the one described in Example 4, obtaining a product which resulted in: MS m/e 364 M^+.

EXAMPLE 9

Preparation of the compound of formula (I) wherein X_1 = S, X_2 = O, R_1 = –$(CH_2)_{13}CH_3$, R_2 = *i*-butyl, and R_3 = R_4 = H [compound (9)]

Compound (9) was prepared following a procedure similar to the one described in Example 3, obtaining a product which resulted in: MS m/e 380 M^+.

EXAMPLE 10

Preparation of the compound of formula (I) wherein $X_1 = X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 =i-butyl, and $R_3 = R_4 = H$ [compound (10)]

Compound (10) was prepared following a procedure similar to the one described in Example 4, obtaining a product which resulted in: MS m/e 364 M^+.

EXAMPLE 11

Preparation of the compound of formula (I) wherein $X_1 = S$, $X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = neopentyl, and $R_3 = R_4 = H$ [compound (11)]

Compound (11) was prepared following a procedure similar to the one described in Example 3, obtaining a product which resulted in: MS m/e 394 M^+.

EXAMPLE 12

Preparation of the compound of formula (I) wherein $X_1 = X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = neopentyl, and $R_3 = R_4 = H$ [compound (12)]

Compound (12) was prepared following a procedure similar to the one described in Example 4, obtaining a product which resulted in: MS m/e 378 M^+.

EXAMPLE 13

Preparation of the compound of formula (I) wherein $X_1 = S$, $X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = 2-phenyl-ethyl, and $R_3 = R_4 = H$ [compound (13)]

Compound (13) was prepared following a procedure similar to the one described in Example 3, obtaining a product which resulted in: MS m/e 428 M^+.

EXAMPLE 14

Preparation of the compound of formula (I) wherein $X_1 = X_2 = O$, $R_1 = -$

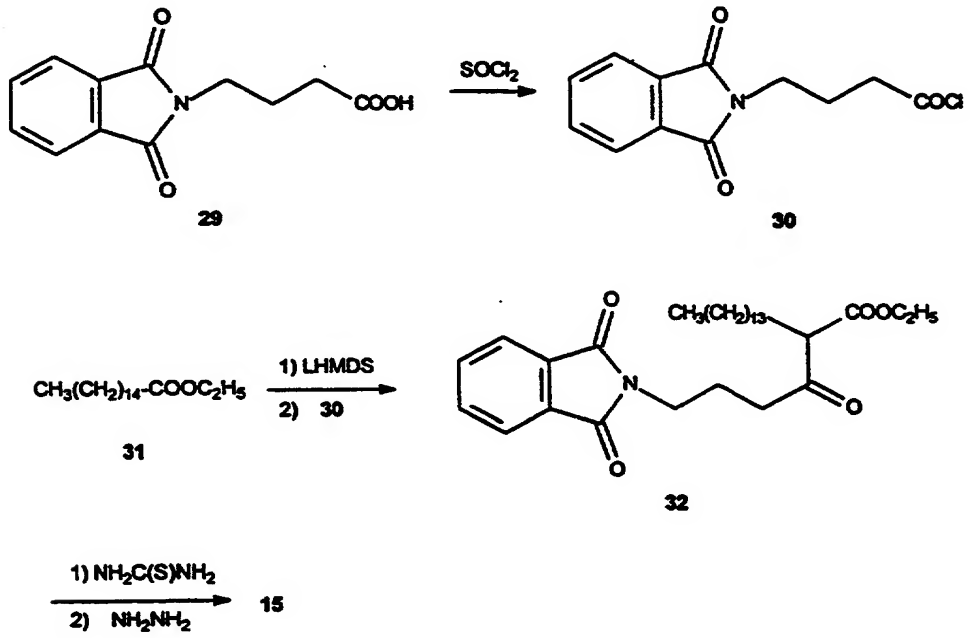
$(CH_2)_{13}CH_3$, R_2 = 2-phenyl-ethyl, and $R_3 = R_4$ = H [compound (14)]

Compound (14) was prepared following a procedure similar to the one described in Example 4, obtaining a product which resulted in: MS m/e 412 M^+.

EXAMPLE 15

Preparation of the compound of formula (I) wherein X_1 =S, X_2 = O, R_1 = – $(CH_2)_{13}CH_3$, R_2 = -$(CH_2)_3NH_2$ and $R_3 = R_4$ = H [compound (15)]

Scheme 1



Compound (15) was prepared following the procedure described in the above Scheme 1.

Synthesis of β-ketoester (32). 2.4 g (10 mmol) of 4-phthalimidoburyric acid (29) (prepared as described in G. Talbot, R. Gaudry, L. Berlinguet *Can. J. Chem.* 1958, 36, 593-596) was dissolved in 7.5 ml of $SOCl_2$ and the mixture was refluxed under nitrogen for 3 hours. Excess of $SOCl_2$ was then removed under a nitrogen flow and the resulting acid chloride (30) was used in the next step without further

purification. Separately, a solution of ethyl palmitate (31) (1.47 g, 5.16 mmol) in anhydrous THF (6.5 ml) was slowly added to a 1.0 M solution of lithium bis(trimethylsilyl)amide (LHMDS) in THF (6.2 ml, 6.2 mmol) cooled at -20 °C and the resulting mixture was stirred for additional 20 minutes. Acid chloride (30) previously prepared as described above, was dissolved in anhydrous THF (10 ml), cooled at -20 °C, and added via cannula to the solution containing (31) and LHMDS at the same temperature. The mixture was stirred at -20 °C for 30 minutes and then at room temperature for 2 hours. The reaction was quenched with a saturated aqueous solution of ammonium chloride and extracted with ethyl acetate. The organic layer was dried over sodium sulfate and concentrated under vacuum. The crude residue was purified by silica gel column chromatography using hexane-ethyl acetate (8:2) as the eluant, to obtain 0.95 g (1.9 mmol, 37% yield) of pure β-ketoester (32) as a colorless oil: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.87 (t, 3H, J = 6.4 Hz), 1.23 (t, 3H, J = 7.3 Hz), 1.24 (bs, 24H), 2.05 (quintet, 2H, J = 7.2 Hz), 2.24-2.34 (m, 4H), 2.51 (t, 2H, J = 7.6 Hz), 3.78 (t, 1H, J = 6.9 Hz), 4.12 (q, 2H, J = 7.1 Hz), 7.69-7.73 (m, 2H), 7.82-7.87 (m, 2H); MS (FAB^+) m/z 500 $(M+H)^+$.

Synthesis of thiouracil (15). β-Ketoester (32) (0.12 g, 0.24 mmol) was dissolved in 2 ml of absolute ethanol. Thiourea (0.024 g, 0.33 mmol) and potassium *t*-butoxyde (0.028 g, 0.25 mmol) were added and the resulting mixture was refluxed for 5 hours. The mixture was then cooled to room temperature and the solvent was removed under vacuum. The residue was treated with 20 ml of water and neutralized with an aqueous solution of acetic acid 0.5 N. The product was extracted with ethyl acetate and the organic layer was washed with brine, dried

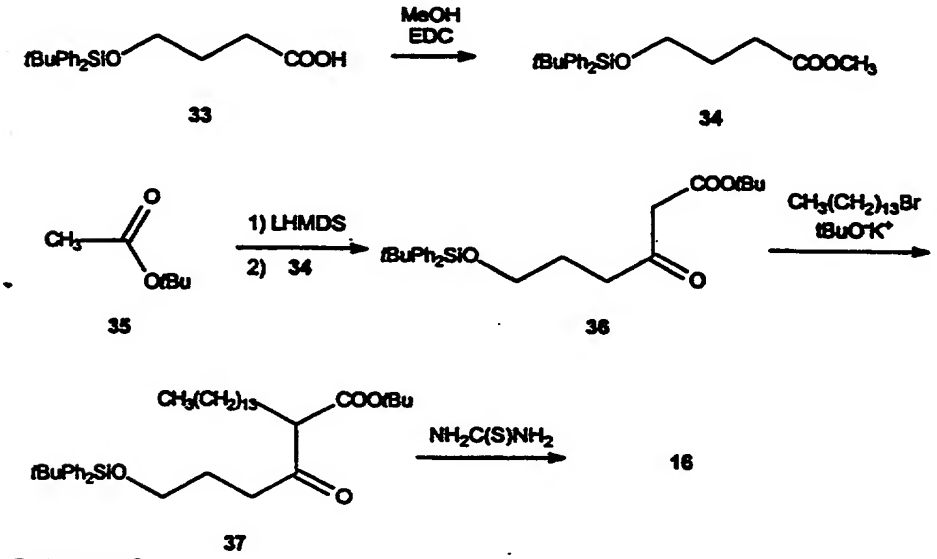
over anhydrous sodium sulfate and concentrated under vacuum. The crude residue was then redissolved in 3 ml of ethanol, treated with 0.06 ml of hydrazine monohydrate (1.3 mmol), and the mixture was refluxed overnight. The resulting suspension was cooled to room temperature. The white solid was collected by filtration, washed with small portions of ethyl acetate, and dried under vacuum, to give 51 mg (0.13 mmol, 54% yield) of product (15): m.p. 123-125 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.88 (t, 3H, J = 6.4 Hz), 1.26 (bs, 24H), 1.77 (m, 2H), 2.29-2.45 (m, 6H), 8.87 (bs, 1H), 9.19 (bs, 1H); MS (FAB$^+$) m/z 381 $(M+H)^+$.

EXAMPLE 16

Preparation of the compound of formula (I) where X_1 =S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = $-(CH_2)_3OSiPh_2t$-Bu and R_3 = R_4 = H [compound (16)]

Compound (16) was prepared following the procedure described in the following

Scheme 2



Scheme 2.

Synthesis of methyl ester (34). A solution of acid (33) (1.15 g, 3.36 mmol) (prepared as in: A.G.M. Barrett, J.A. Flygare *J. Org. Chem.* **1991**, *56*, 638-642) in methanol (25 ml) was treated with 1.62 g (8.44 mmol) of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC). The resulting solution was stirred under nitrogen at room temperature for 3.5 hours. The solvent is then removed under vacuum and the residue was diluted with chloroform (100 ml) and water (50 ml). The organic layer was washed with brine, dried over sodium sulfate and concentrated under vacuum. The crude residue was purified by silica gel column chromatography using hexane-ethyl acetate (9:1) as the eluant, to obtain 0.59 g (1.6 mmol, 49% yield) of pure ester (34) as a colorless oil: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.05 (s, 9H), 1.88 (tt, 2H, J = 7.7, 5.9 Hz), 2.47 (t, 2H, J = 7.5 Hz), 3.66 (s, 3H), 3.68 (t, 2H, J = 6.0 Hz), 7.37-7.42 (m, 6H), 7.63-7.68 (m, 4H).

Synthesis of β-ketoester (36). A solution of *t*-butyl acetate (35) (4.24 g, 36.5 mmol) in anhydrous THF (40 ml) previously cooled at -78 °C was added drop by drop via cannula under argon to a 1M solution of LHMDS in THF (51.5 ml, 51.5 mmol). To the resulting solution, previously stirred at the same temperature for 30 minutes, was added drop by drop via cannula another solution of methyl ester (34) (4.07 g, 11.4 mmol) in anhydrous THF (20 ml) at -78 °C. The reaction mixture was stirred under argon for 20 minutes at the same temperature, and then 3 more hours at room temperature. The reaction was quenched with 400 ml of saturated aqueous solution of ammonium chloride and extracted with diethyl ether (2 x 300 ml). The organic layer was dried over anhydrous sodium sulfate and concentrated under vacuum. The crude residue was purified by silica gel column chromatography using hexane-diethyl ether (8:2) as the eluant, to obtain 2.8 g (6.4 mmol, 56%

yield) of pure β-ketoester (**36**) as a colorless oil: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.04 (s, 9H), 1.46 (s, 9H), 1.84 (quintet, 2H, *J* = 6.7 Hz), 2.66 (t, 2H, *J* = 7.3 Hz), 3.34 (s, 2H), 3.67 (t, 2H, *J* = 6.0 Hz), 7.37-7.43 (m, 6H), 7.62-7.67 (m, 4H); MS (FAB^+) m/z 441 $(M+H)^+$, 385 $(M+H\text{-isobutene})^+$.

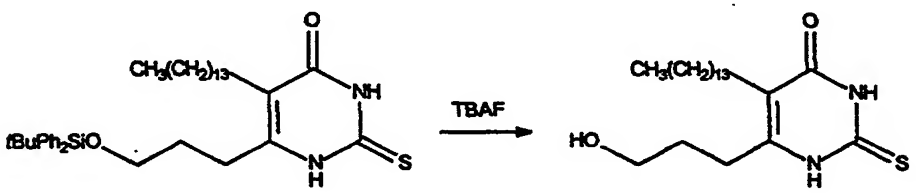
Synthesis of alkylated β-ketoester (**37**). A solution of β-ketoester (**36**) (2.79 g, 6.34 mmol) in anhydrous 1,2-dimethoxyethane (DME) (17 ml) was added to a solution of potassium *tert*-butoxide (0.85 g, 6.97 mmol) in anhydrous DME (7 ml). The resulting solution was stirred at room temperature for 20 minutes, after which time 1.7 ml (1.6 g, 5.7 mmol) of 1-bromotetradecane were added. The reaction mixture was stirred at 80 °C for 2 hours. The reaction was quenched with 150 ml of a saturated aqueous solution of ammonium chloride and extracted with diethylether (2 x 300 ml). The organic layer was washed with brine, dried over sodium sulfate and concentrated under vacuum. The crude residue was purified by silica gel column chromatography using hexane-diethyl ether (9:1) as the eluant, to obtain 1.16 g (1.82 mmol, 32% yield) of pure mono-alkylated product (**37**) as a colorless oil: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.88 (t, 3H, *J* = 6.2 Hz), 1.04 (s, 9H), 1.25 (bs, 24H), 1.43 (s, 9H), 1.76-1.89 (m, 4H), 2.64 (td, 2H, *J* = 7.3, 4.4 Hz), 3.13 (t, 1H, *J* = 7.3 Hz), 3.66 (t, 2H, *J* = 6.0 Hz), 7.34-7.43 (m, 6H), 7.62-7.67 (m, 4H); MS (FAB^+) m/z 581 $(M+H\text{-isobutene})^+$, 563 $(M\text{-}t\text{BuO})^+$.

Synthesis of thiouracil (**16**). A solution containing alkylated β-ketoester (**37**) (1.16 g, 1.82 mmol) in absolute ethanol (24 ml) in a screw-cap sealed vial was treated first with 0.19 g (2.6 mmol) of thiourea and then with 0.25 g (2.0 mmol) of potassium *tert*-butoxide. The resulting solution was stirred at 100 °C for 6 hours. The solvent was then removed under vacuum. The residue was diluted with water

and neutralized to pH = 6-7 with 0.5 N acetic acid. The product was extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate and concentrated under vacuum. The crude residue was purified by silica gel column chromatography using hexane-diethyl ether (8:2) as the eluant, to obtain 0.52 g (0.84 mmol, 46% yield) of pure thiouracil product (16) as a colorless oil: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.87 (t, 3H, *J* = 6.2 Hz), 1.10 (s, 9H), 1.24 (bs, 24 H), 1.74 (quintet, 2H, *J* = 6.8 Hz), 2.34 (t, 2H, *J* = 7.4 Hz), 2.60 (t, 2H, *J* = 7.5 Hz), 3.74 (t, 2H, *J* = 5.8 Hz), 7.40-7.46 (m, 6H), 7.66-7.70 (m, 4H), 9.29 (bs, 1H), 9.55 (bs, 1H); MS (FAB$^+$) m/z 547 $(M+H-NHC(S)NH)^+$.

EXAMPLE 17

Preparation of the compound of formula (I) where X_1 =S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = $-(CH_2)_3OH$ and R_3 = R_4 = H [compound (17)]



16 17

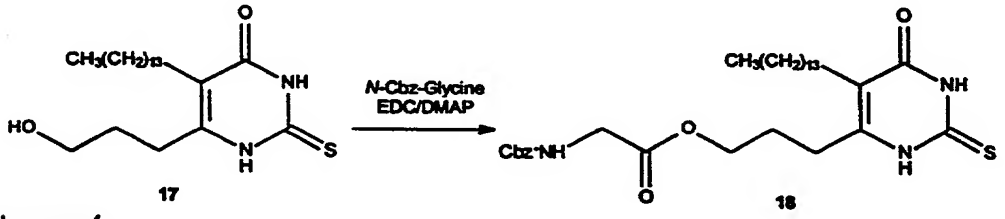
Scheme 3

According to the above Scheme 3 the silyl ether **16** (0.16 g, 0.26 mmol) was treated with 0.8 ml of a 1M solution of tetrabutylammonium fluoride (TBAF) in THF (0.8 mmol) under argon at room temperature for 2 hours. The solvent was then removed under vacuum and the residue was extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate and concentrated under vacuum. The crude residue was purified by silica gel column chromatography using hexane-ethyl acetate (1:1) as the eluant, to obtain 0.079 g (0.21 mmol, 81% yield) of pure deprotected alcohol (17) as a white solid: m.p. 128-

130 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.88 (t, 3H, *J* = 6.7 Hz), 1.25 (bs, 24H), 1.91 (quintet, 2H, *J* = 6.1 Hz), 2.37 (pseudo t, 2H, *J* = 7.4 Hz), 2.67 (pseudo t, 2H, *J* = 6.3 Hz), 3.84 (t, 2H, *J* = 5.6 Hz), 9.16 (bs, 1H), 10.52 (bs, 1H); MS (EI, 70 eV) m/z 382 $(M)^+$, 365 $(M\text{-}OH)^+$, 323 $(M\text{-}NHC{=}S)^+$.

EXAMPLE 18

Preparation of the compound of formula (I) where X_1 =S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = $-(CH_2)_3OC(O)CH_2NH$-Cbz and R_3 = R_4 = H [compound (18)]



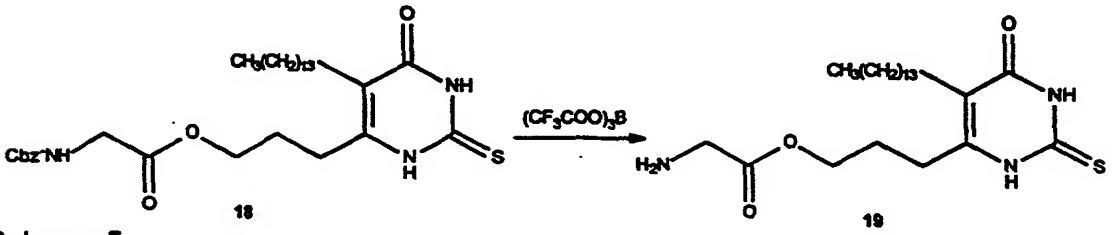
Scheme 4

According to scheme 4 a solution of the alcohol (17) (0.038 g, 0.099 mmol) in anhydrous THF (2.5 ml) was sequentially treated with 0.031 g (0.15 mmol) of *N*-carbobenzyloxyglycine (*N*-Cbz-Gly), 0.034 g (0.18 mmol) of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC), and 0.0012 g (0.0096 mmol) of 4-(dimethylamino)pyridine (DMAP). The mixture was stirred at room temperature for 5 hours under argon. The solvent was removed under vacuum and the residue was purified by silica gel column chromatography using hexane-ethyl acetate (1:1) as the eluant, to obtain 0.052 g (0.091 mmol, 92% yield) of product (18) as a thick syrup: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.87 (t, 3H, J = 6.6 Hz), 1.25 (bs, 24H), 1.91 (m, 2H), 2.31 (t, 2H, J = 7.7 Hz), 2.47 (t, 2H, J = 7.7 Hz), 4.07 (d, 2H, J = 5.9 Hz), 4.27 (t, 2H, J = 5.2 Hz), 5.24 (s, 2H), 5.52 (t, 1H, J = 5.8 Hz), 7.31-7.38 (m, 5H), 10.09 (bs, 1H), 10.85 (bs 1H); MS (FAB^+) m/z 574

$(M+H)^+$, 532 $(M-C(S)+H)^+$.

EXAMPLE 19

Preparation of the compound of formula (I) where X_1 =S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = $-(CH_2)_3OC(O)CH_2NH_2$ and R_3 = R_4 = H [compound (19)]

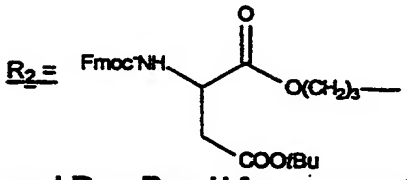


Scheme 5

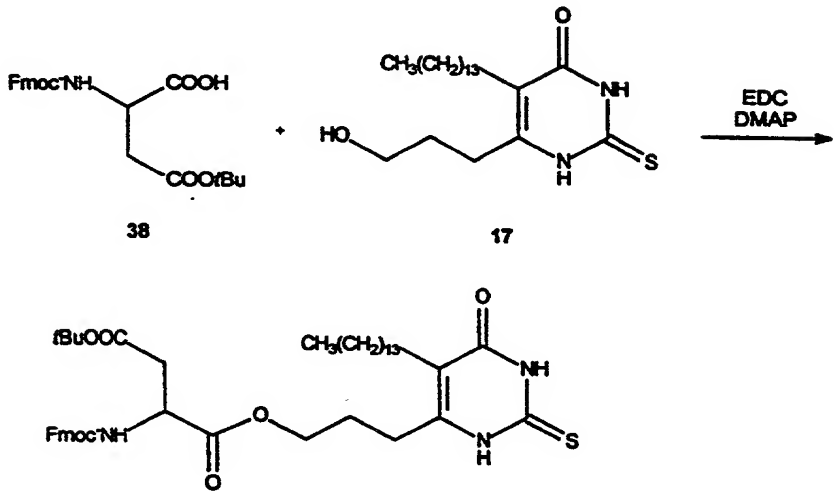
According to the above scheme 5 a solution of Cbz-protected compound (18) (0.032 g, 0.055 mmol) in trifluoroacetic acid (1 ml) was treated with 0.22 mmol of freshly prepared boron tris(trifluoroacetate) (prepared as reported in: J. Pless, W. Bauer *Angew. Chem. Int. Ed.* **1973**, *12*, 147-148) at 0 °C under argon. The mixture was stirred for 1 hour at the same temperature and overnight at room temperature. The solvent was removed under vacuum and the residue was purified by silica gel column chromatography using a mixture dichloromethane : acetone 7 : 3 as the eluant, to obtain 0.020 g (0.045 mmol, 82% yield) of product (19) as a thick syrup: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.87 (t, 3H, *J* = 6.4 Hz), 1.25 (bs, 24H), 1.72 (m, 2H), 2.32 (m, 2H), 2.63 (m, 2H), 3.61 (t, 2H, *J* = 7.0 Hz), 4.30 (t, 2H, *J* = 6.6 Hz); MS (FAB^+) m/z 365 $(M-NHC(S)NH)^+$.

EXAMPLE 20

Preparation of the compound of formula (I) where X_1=S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$,



and R_3 = R_4 = H [compound (20)]



Scheme 6

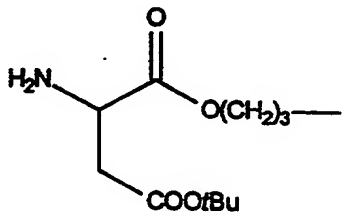
According to the above scheme 6 a solution of alcohol (**17**) (0.120 g, 0.314 mmol) in anhydrous THF (10 ml) was treated sequentially with *N*-(9-Fluorenylmethoxycarbonyl)-L-aspartic acid *tert*-butyl ester (**38**) (0.194 g, 0.471 mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC) (0.108 g, 0.562 mmol) and 4-(dimethylamino)pyridine (DMAP) (0.0077 g, 0.063 mmol). The mixture was stirred under argon at room temperature for 5 hours. The solvent was removed under vacuum and the residue was purified by silica gel column chromatography (hexane - ethyl acetate 1:1) to afford 0.24 g (0.31 mmol, 98 % yield) of product (**20**) as a syrup: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.87 (t, 3H, *J* = 6.3 Hz), 1.25 (bs, 24H), 1.46 (s, 9H), 1.93 (m, 2H), 2.30 (m, 2H), 2.49 (m, 2H),

2.80 (dd, 1H, J = 16.6, 4.8 Hz), 2.93 (dd, 1H, J = 16.6, 5.0 Hz), 4.24-4.33 (m, 2H), 4.48-4.54 (m, 2H), 4.67-4.72 (m, 1H), 5.97 (d, 1H), 7.29-7.43 (m, 5H), 7.62 (d, 2H, J = 7.2 Hz), 7.76 (d, 2H, J = 7.2 Hz), 9.59 (bs, 1H), 10.58 (bs, 1H).

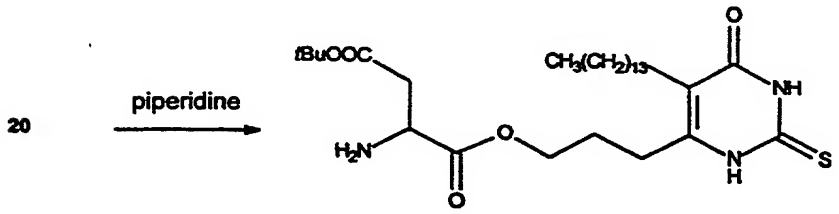
EXAMPLE 21

Preparation of the compound of formula (I) where X_1 =S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$,

R_2 =



and R_3 = R_4 = H [compound (21)]



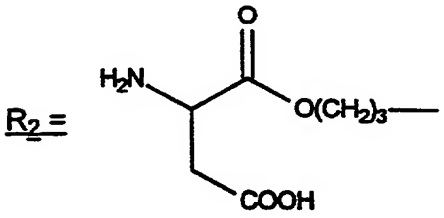
21

Scheme 7

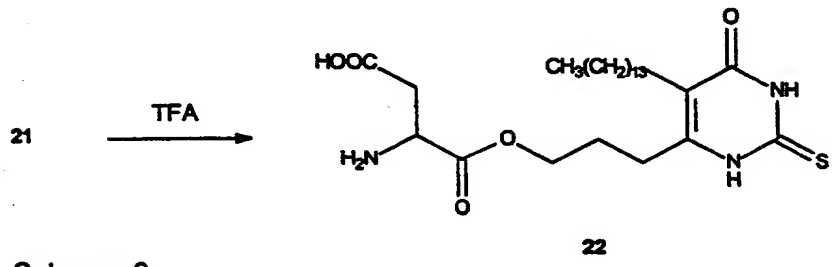
According to the above scheme 7 a solution of Fmoc-protected product (**20**) (0.120 g, 0.155 mmol) in anhydrous dichloromethane (5 ml) was treated with 0.020 g of piperidine (0.23 mmol). The mixture was stirred at room temperature for 30 minutes. The solvent was removed under vacuum and the residue was purified by silica gel column chromatography (hexane - ethyl acetate 3:7) to afford 0.040 g (0.072 mmol, 47 % yield) of product (**20**) as a syrup: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.87 (t, 3H, J = 6.6 Hz), 1.25 (bs, 24H), 1.46 (s, 9H), 1.94 (m, 2H), 2.33 (t, 2H, J = 7.2 Hz), 2.56 (t, 2H, J = 7.7 Hz), 2.76 (d, 2H, J = 5.9 Hz), 3.94 (t, 1H, J = 5.8 Hz), 4.21-4.30 (m, 2H).

EXAMPLE 22

Preparation of the compound of formula (I) where X_1=S, X_2 = O, R_1 = –$(CH_2)_{13}CH_3$,



and R_3 = R_4 = H [compound (22)]

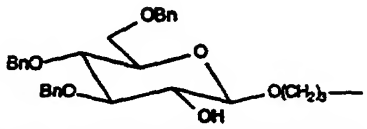


Scheme 8

According to the above scheme 8 the *tert*-Butyl ester (21) (0.020 g, 0.040 mmol) was treated with 0.2 ml of a 1:1 mixture of trifluoroacetic acid and dichloromethane. The mixture was stirred at room temperature for 1 hour. The solvent was removed under vacuum and the residue was purified by silica gel column chromatography (acetone - methanol, variable ratios from 100:0 to 50:50) to afford 0.012 g (0.021 mmol, 54 % yield) of product (20) as a syrup: ^{1}H NMR (CD_3OD, 200 MHz) δ 0.89 (t, 3H, *J* = 6.8 Hz), 1.29 (bs, 24H), 1.97 (m, 2H), 2.35 (m, 2H), 2.57 (m, 2H), 2.82 (m, 2H), 4.16-4.44 (m, 3H).

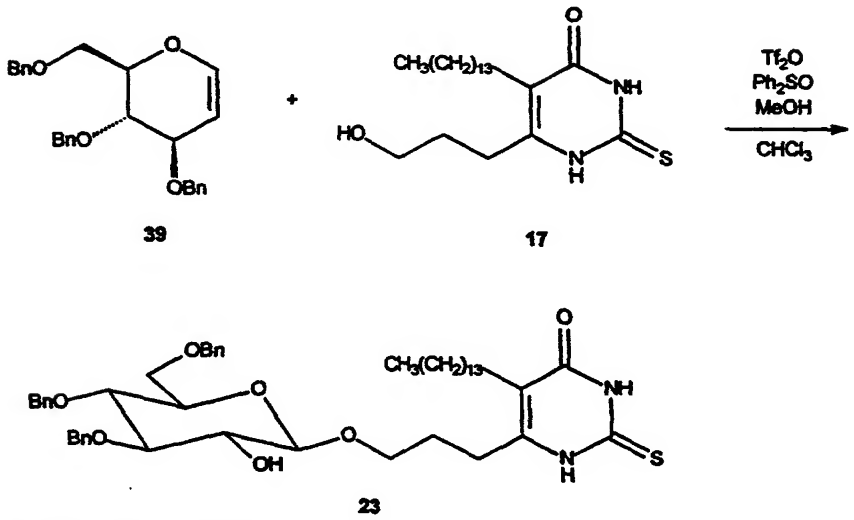
EXAMPLE 23

Preparation of the compound of formula (I) where X_1 =S, X_2 = O, R_1 = –$(CH_2)_{13}CH_3$,



and R_3 = R_4 = H [compound (23)]

Scheme 9



wherein Bn is benzyl.

Glucose derivative (**23**) was prepared following a general procedure for direct glycosilation of alcohols with glucal donor (**39**) (as reported in: V. Di Bussolo, Y.-J. Kim, D.Y. Gin *J. Am. Chem. Soc.* **1998**, *120*, 13515-13516), as reported above in scheme 9.

Trifluoromethanesulfonic anhydride (Tf_2O) (0.030 ml, 0.18 mmol) was added to a solution of tri-O-benzil-D-glucal (**39**) (0.050 g, 0.12 mmol), diphenylsulfoxide (0.073 g, 0.36 mmol) and 2,4,6-tri-*t*-butylpyridine (0.104 g, 0.42 mmol) in dry chloroform (5 ml) (distilled over P_2O_5) at - 40 °C. The reaction mixture was stirred at this temperature for 1 hour. Methanol (0.005 ml, 0.12 mmol) and triethylamine

(0.050 ml, 0.36 mmol) were added sequentially at -40 °C and the reaction mixture was stirred at this temperature for 30 minutes, then at 0 °C for 1 hour and at room temperature for 1 hour. A solution of alcohol derivative (17) (0.065 g, 0.17 mmol) in dry chloroform (4 ml) was added at 0 °C, via cannula. Zinc chloride (0.24 ml, 1.0 M in diethyl ether, 0.24 mmol) was added at the same temperature, then the temperature was slowly warmed to room temperature and the reaction mixture stirred at this temperature for 12 hours. The reaction was diluted with chloroform (15 ml) and washed sequentially with saturated aqueous sodium bicarbonate solution (2 x 15 ml) and a saturated aqueous solution of sodium chloride (15 ml). The organic layer was dried (Na_2SO_4) and concentrated, the residue was purified by silica gel column chromatography (hexane-ethyl acetate 6:4) to afford product (23) (0.055 g, 0.067 mmol, 56% yield) as a colourless oil: ^1H NMR ($CDCl_3$) δ 0.87 (t, 3H, *J* = 6.3 Hz), 1.25 (bs, 24 H), 1.88 (quintet, 2H, *J* = 6.4 Hz), 2.44 (pseudo *t*, 2H, *J* = 7.5 Hz), 2.65 (t, 2H, *J* = 6.6 Hz), 3.70-3.66 (m, 8H), 4.47 (d, 1H, *J* = 10.6 Hz), 4.52 (d, 1H, *J* = 12.1 Hz), 4.65 (d, 1H, *J* = 12.1 Hz), 4.80 (d, 1H, *J* = 10.8 Hz), 4.86 (d, 1H, *J* = 11.4 Hz), 4.92 (d, 1H, *J* = 11.2 Hz), 5.12 (d, 1H, *J* = 9.2 Hz), 7.09-7.35 (m, 15H), 9.61 (bs, 1H), 11.29 (bs, 1H); MS (FAB^+) m/z 815 $(M+H)^+$.

EXAMPLE 24

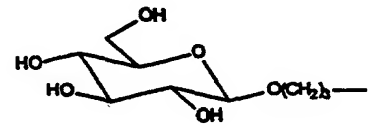
Preparation of the compound of formula (I) where X_1 = S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = ethyl, R_3 = $-CH_2COOC_2H_5$, and R_4 = H [compound (24)]

Anhydrous $(NH_4)_2SO_4$ (0.0013 g, 0.011 mmol) and 1,1,1,3,3,3-hexamethyldisilazane (HMDS) (0.75 ml, 3.41 mmol) were added, under argon atmosphere, to compound (3) (0.05 g, 0.14 mmol). The resulting suspension was heated at 130 °C and stirred at this temperature for 6 hours. The mixture was then

concentrated at room temperature under a flux of argon. Anhydrous THF (3 ml) was added, and the resulting solution was stirred at - 45°C. Trimethylsilyl triflate (TMS triflate) (0.03 ml, 0.145 mmol) and ethyl bromoacetate (0.046 g, 0.027 mmol) were sequentially added and the mixture was stirred at - 45 °C for 3 hours, then at room temperature for 1 hour. Saturated aqueous $NaHCO_3$ (3 ml) was added and THF was removed under vacuum. The residue was diluted with H_2O (20 ml) and extracted with ethyl acetate (3 x 10 ml). The organic layer was dried with Na_2SO_4 anhydrous, and concentrated to dryness. The residue was purified by semi-preparative thin-layer column chromatography (hexane/ethyl acetate 7:3) to afford product (24) (0.010 g, 0.023 mmol, 16% yield) as a colourless oil: ^1H NMR ($CDCl_3$, 200 MHz) δ 0.87 (t, 3H, J = 6.6 Hz), 1.17 (t, 3H, J = 7.2 Hz), 1.25-1.43 (m, 27H), 2.44 (pseudo t, 2H, J = 7.2 Hz), 2.54 (t, 2H, J = 7.5 Hz), 3.91 (s, 2H); 4.21 (q, 2H, J = 7.3 Hz), 10.88 (bs, 1H);); MS (FAB^+) m/z 439 $(M+H)^+$.

According to procedures analogues to those above reported, the following compounds of formula (I) were prepared:

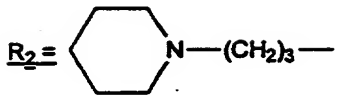
- compound (I) wherein X_1 =S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$,

R_2 = 

and R_3 = R_4 = H [compound (25)];

- compound (I) wherein X_1 =S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = $-(CH_2)_3Br$ and R_3 = R_4 = H [compound (26)];

- compound (I) wherein X_1 =S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$,



and $R_3 = R_4 = H$ [compound (27)];

- compound (I) wherein $X_1 = S$, $X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, $R_2 = -(CH_2)_3N(C_2H_5)_3^+Br^-$ and $R_3 = R_4 = H$ [compound (28)].

CYTOTOXICITY TEST

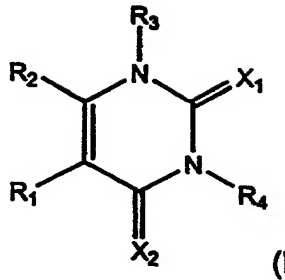
The cytotoxicity of the compounds synthesized 1 - 28 was assessed using a human leukemia cell line called CCRF/CEM. The CCRF/CEM cells were cultured in a culture medium containing RPMI 1640 (90%), bovine fetal sera (10%) and interleukin-2 (100 U/ml). The cytotoxicity assay was performed on 104 CCRF/CEM cells seeded in 35 mm wells in 2 ml of culture medium. The cells were treated with the compounds under consideration for 72 hours and at the end of the period of exposure their number was counted and compared with that of control cells treated with C_2-ceramide in order to establish the percentage of growth inhibition. The concentration capable of inhibiting 50% of cell growth was calculated by non-linear regression of the experimental data as described in M. Macchia, N. Jannitti, G.B. Gervasi, R. Danesi, *J.Med Chem*, (1996) 39 (7): 1352-1356.

The resulting values of IC_{50} expressed in μM are given in the following table:

Compound	IC_{50} (μM)
controls	31.6
(3)	1.7
(4)	6.3
(6)	0.97
(9)	13.2
(10)	8.7
(11)	20
(12)	29.1
(13)	20.7
(14)	15.6

Claims

1. Compounds of general formula (I)



(I)

where

X_1 and X_2 are selected between O and S;

R_1 and R_2 are selected between $-(CH_2)_{13}CH_3$ and alkyl or alkylene groups with from 2 to 6 carbon atoms, linear or branching, unsubstituted or substituted with one or more substituents selected among aromatic, primary, secondary and tertiary aminic, quaternary ammonium, carboxylic, hydroxylic, polyoxyalkyl and ethereal groups, aminoacids, halogen atoms or saccharidic portions, providing that between R_1 and R_2 only one is always $-(CH_2)_{13}CH_3$,

R_3 and R_4 are selected between H and alkyl or alkylene groups with from 2 to 6 carbon atoms, linear or branching, unsubstituted or substituted with one or more substituents selected among aromatic, primary, secondary and tertiary aminic, quaternary ammonium, carboxylic, hydroxylic, polyoxyalkyl and ethereal groups, aminoacids, halogen atoms or saccharidic portions.

2. The compounds of general formula (I) according to claim 1, where:

$X_1 = S$, $X_2 = O$, R_1 = ethyl, $R_2 = -(CH_2)_{13}CH_3$, and $R_3 = R_4 = H$ (compound **1**);

$X_1 = X_2 = O$, R_1 = ethyl, $R_2 = -(CH_2)_{13}CH_3$, and $R_3 = R_4 = H$ (compound **2**);

$X_1 = S$, $X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = ethyl, and $R_3 = R_4 = H$ (compound **3**);

$X_1 = X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = ethyl, and $R_3 = R_4 = H$ (compound **4**);

X_1 = S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = *n*-propyl, and R_3 = R_4 = H (compound **5**);

X_1 = X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = *n*-propyl, and R_3 = R_4 = H (compound **6**);

X_1 = S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = *n*-butyl, and R_3 = R_4 = H (compound **7**);

X_1 = X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = *n*-butyl, and R_3 = R_4 = H (compound **8**);

X_1 = S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = *i*-butyl, and R_3 = R_4 = H (compound **9**);

X_1 = X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = *i*-butyl, and R_3 = R_4 = H (compound **10**);

X_1 = S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = neopentyl, and R_3 = R_4 = H (compound **11**);

X_1 = X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = neopentyl, and R_3 = R_4 = H (compound **12**);

X_1 = S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = 2-phenyl-ethyl, and R_3 = R_4 = H (compound **13**);

X_1 = X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = 2-phenyl-ethyl, and R_3 = R_4 = H (compound **14**);

X_1 = S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2= $-(CH_2)_3NH_2$, and R_3 = R_4 = H (compound **15**);

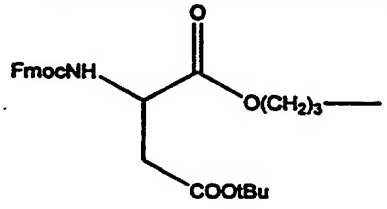
X_1 =S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = $-(CH_2)_3OSiPh_2t$-Bu, and R_3 = R_4 = H (compound **16**);

X_1 = S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = $-(CH_2)_3OH$, and R_3 = R_4 = H (compound **17**);

X_1 =S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = $-(CH_2)_3OC(O)CH_2NH$-Cbz, and R_3 = R_4 = H (compound **18**);

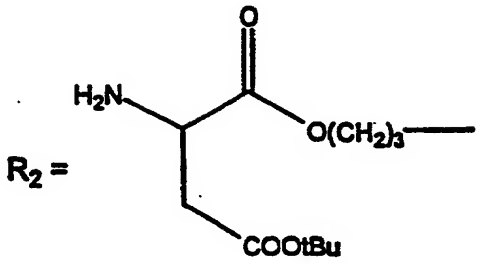
X_1 =S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = $-(CH_2)_3OC(O)CH_2NH_2$, and R_3 = R_4 = H (compound **19**);

X_1 =S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 =



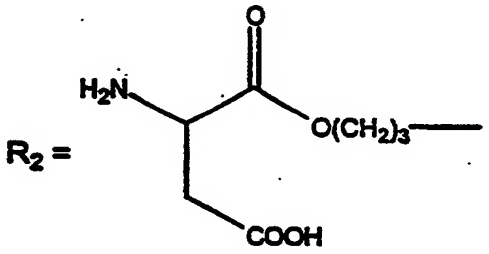
and $R_3 = R_4 = H$ (compound **20**);

$X_1 = S$, $X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$



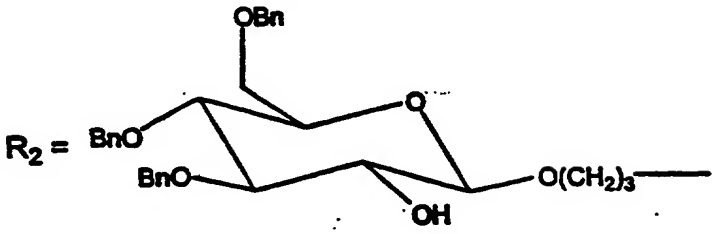
and $R_3 = R_4 = H$ (compound **21**);

$X_1 = S$, $X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$



and $R_3 = R_4 = H$ (compound **22**);

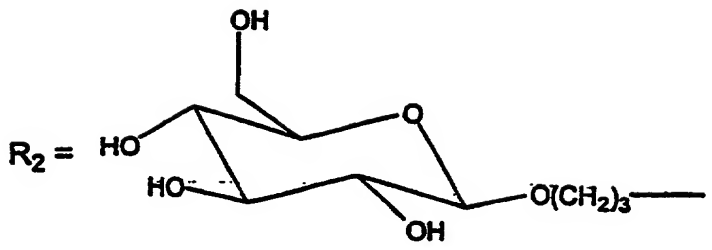
$X_1 = S$, $X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$



wherein Bn is benzyl and $R_3 = R_4 = H$ (compound **23**);

$X_1 = S$, $X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = ethyl, $R_3 = -CH_2COOC_2H_5$, and $R_4 = H$ (compound **24**);

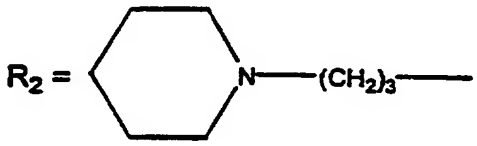
$X_1 = S$, $X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$



and $R_3 = R_4 = H$ (compound **25**);

$X_1 = S$, $X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, $R_2 = -(CH_2)_3Br$, and $R_3 = R_4 = H$ (compound **26**);

$X_1 = S$, $X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$



and $R_3 = R_4 = H$ (compound **27**);

$X_1 = S$, $X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, $R_2 = -(CH_2)_3N(C_2H_5)_3^+Br^-$, and $R_3 = R_4 = H$ (compound **28**).

3. The compounds of general formula (I) according to claim 1, where:

$X_1 = S$, $X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = ethyl, and $R_3 = R_4 = H$ (compound **3**);

$X_1 = X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = ethyl, and $R_3 = R_4 = H$ (compound **4**);

$X_1 = X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = *n*-propyl, and $R_3 = R_4 = H$ (compound **6**);

$X_1 = X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = *i*-butyl, and $R_3 = R_4 = H$ (compound **10**).

4. Pharmaceutical preparations including as their active ingredient at least one of the compounds of the general formula (I) described in claims 1-3, and/or their pharmaceutically acceptable derivatives or salts, together with excipients and/or

diluents.

5. Use of the compounds of the general formula (I) described in claims 1-3 for the preparation of pharmaceutical formulations.

6. The use according to claim 5, for the preparation of pharmaceutical formulations for use in the treatment of tumours.

PTO/SB/01 (10-01)
Approved for use through 10/31/2002. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)

☑ Declaration Submitted with Initial Filing **OR** ☐ Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16 (e)) required)

Attorney Docket Number	322/B0437
First Named Inventor	MACCHIA
COMPLETE IF KNOWN	
Application Number	
Filing Date	
Art Unit	
Examiner Name	

As the below named inventor, I hereby declare that:

My residence, mailing address, and citizenship are as stated below next to my name.

I believe I am the original and first inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled:

CERAMIDE ANALOGS, PROCESS FOR THEIR PREPARATION AND THEIR USE AS ANTITUMOR AGENTS

(Title of the Invention)

the specification of which

☐ is attached hereto

OR

☑ was filed on (MM/DD/YYYY) 07/21/2000 as United States Application Number or PCT International Application Number PCT/EP00/07023 and was amended on (MM/DD/YYYY) _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or (f), or 365(b) of any foreign application(s) for patent, inventor's or plant breeder's rights certificate(s), or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent, inventor's or plant breeder's rights certificate(s), or any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached? YES	Certified Copy Attached? NO
FI99A000169	IT	07/22/1999	☐	☐	☑
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

DECLARATION — Utility or Design Patent Application

Direct all correspondence to: ☐ Customer Number or Bar Code Label [ ] OR ☑ Correspondence address below

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Name

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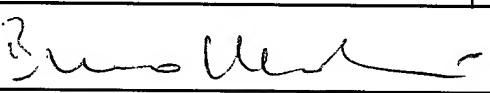
I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

NAME OF SOLE OR FIRST INVENTOR : ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any]) Bruno	Family Name or Surname Macchia

100

Inventor's Signature 	Date 15.01.02

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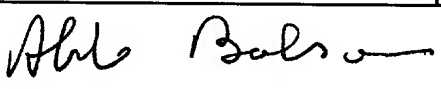
NAME OF SECOND INVENTOR: ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any]) Aldo	Family Name or Surname Balsamo

2°°

Inventor's Signature 	Date 15.01.02

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City	**State**	**ZIP**	**Country**

☑ Additional inventors are being named on the 1 supplemental Additional Inventor(s) sheet(s) PTO/SB/02A attached hereto.

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DECLARATION

ADDITIONAL INVENTOR(S)
Supplemental Sheet
Page 1 of 1

3 00

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor		
Given Name: Marco	Family Name or Surname: Macchia		
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4 00

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor		
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Inventor's Signature: [signature]		Date: 15/01/02	
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City: Pisa	State:	ZIP: I-56100	Country: IT

5 00

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor		
Given Name: Romano	Family Name or Surname: Danesi		
Inventor's Signature: [signature]		Date: 15/01/02	
Residence: City: Livorno ITX	State:	Country: IT	Citizenship: IT
Mailing Address: Viale Ugo Foscolo, 99			
Mailing Address:			
City: Livorno	State:	ZIP: I-57100	Country: IT

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